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# Understanding the mechanisms of efficacy of fecal microbiota transplant in treating recurrent *Clostridioides difficile* infection and beyond: the contribution of gut microbial-derived metabolites

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ADDENDUM



## Understanding the mechanisms of efficacy of fecal microbiota transplant in treating recurrent *Clostridioides difficile* infection and beyond: the contribution of gut microbial-derived metabolites

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### ABSTRACT

Fecal microbiota transplant (FMT) is a highly-effective therapy for recurrent *Clostridioides difficile* infection (rCDI), and shows promise for certain non-CDI indications. However, at present, its mechanisms of efficacy have remained poorly understood. Recent studies by our laboratory have noted the particular key importance of restoration of gut microbe-metabolite interactions in the ability of FMT to treat rCDI, including the impact of FMT upon short chain fatty acid (SCFAs) and bile acid metabolism. This includes a significant impact of these metabolites upon the life cycle of *C. difficile* directly, along with potential postulated additional benefits, including effects upon host immune response. In this *Addendum*, we first present an overview of these recent advancements in this field, and then describe additional novel data from our laboratory on the impact of FMT for rCDI upon several gut microbial-derived metabolites which had not previously been implicated as being of relevance.

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Gut microbiome; metabonomics; fecal microbiota transplant; *Clostridioides difficile* infection; bile acids; short chain fatty acids; trimethylamine

## 1. Introduction


Fecal microbiota transplant (FMT) is widely-recognized as a highly-efficacious treatment for recurrent/refractory *Clostridioides difficile* infection (rCDI) that has not responded to conventional pharmacological therapy, such as fidaxomicin or vancomycin.<sup>1,2</sup> There is also growing interest in the potential application of FMT for a range of non-CDI indications.<sup>3</sup> However, there are certain clear drawbacks related to the use of FMT in its present form, including the complex regulation associated with its use, the potential need for invasive administration (i.e. endoscopy – although capsulized FMT is

increasingly available), and the risk of transmission of infection from donor to recipient. The latter point is particularly salient, given a recent report of multi-drug resistant *E. coli* being transmitted via FMT (to an immunosuppressed patient without rCDI) with a resultant patient death.<sup>4</sup> Furthermore, there are currently no established biological means for matching stool donor with recipient, despite certain proposals.<sup>5</sup> As such, deconvoluting the mechanisms of efficacy of FMT – and exploiting this knowledge to develop novel targeted microbiome therapeutics, or to better match donor and recipient – is a major clinical priority.

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FMT for rCDI rapidly restores a gut microbiota, that has been hugely disrupted by recurrent antibiotic therapy, back toward pre-morbid composition and diversity, resembling that of healthy donors.<sup>6,7</sup> Proof-of-concept studies have further inferred the central role of restoration of gut bacteria specifically in the efficacy of FMT, demonstrating that either a healthy donor-derived defined community of commensal bacteria<sup>8</sup> or fractionated spores from ethanol-shocked donor stool<sup>9</sup> may have comparable efficacy to conventional FMT in treating rCDI. However, a more recent pilot study demonstrated that sterile filtered donor stool (filtered through a 0.2 µm filter, small than the size of a bacterium) also effectively caused sustained remission from rCDI.<sup>10</sup> This study raised the intriguing possibility that soluble factors – rather than intact bacteria *per se* – were key mediators for the efficacy of FMT.

Among such possible factors, non-bacterial gut microbiota components have been one area of focus. Several studies have described that the stool donor virome or mycobiome is associated with the efficacy of FMT for rCDI, and that these undergo rapid changes in the stool of rCDI patients treated successfully with FMT.<sup>11–14</sup> However, the significance of these changes as a potential mechanism of efficacy of FMT remains unclear. For example, given the narrow range that bacteriophages possess, any anti-*C. difficile* lytic phages in the gut virome post-FMT would have had to have originated from *C. difficile* in the donor virome; given that *C. difficile* carriage is a near universal exclusion criterion for acceptance as an FMT donor, this would be very unlikely in practice. Similarly, given the established relationship between antimicrobial treatment and *Candida* overgrowth within the gut, any changes in gut mycobiome profiles post-FMT might only represent proxies of gut bacterial alterations. Consequently, the specific contribution of bacteriophages and fungi to the efficacy of FMT remains undefined.

A further key area of interest has been whether a potential mechanism of FMT may be through the restoration of microbial metabolites, or of co-metabolites derived from interaction between the gut microbiota and host. This hypothesis has been the focus of much recent research from our laboratory. In the coming sections, we first summarize our recently-published work in this area, before introducing additional novel data.

## 2. Impact of FMT for rCDI upon gut microbial metabolites: recently-discovered areas

### 2.1. Bile acid metabolism

Different bile acids have varied effects upon the ability of *C. difficile* to undergo germination or vegetative growth *in vitro*. Specifically, the conjugated primary bile taurocholic acid (TCA) promotes spore germination of *C. difficile* (with glycine functioning as pro-germinant);<sup>15,16</sup> in contrast, secondary bile acids (including deoxycholic acid (DCA)) inhibit the vegetative growth and toxin activity of the bacterium.<sup>15,17</sup> In mammals, conversion from primary to secondary bile acids occurs within the distal gut, undertaken by several enzymes produced by the gut microbiota, but not by mammals. The two principle enzymatic processes are a first step mediated by *bile salt hydrolase* (*BSH*; which hydrolyzes the glycine or taurine group from conjugated bile acids, aka choloylglycine hydrolase (EC 3.5.1.24)) and a second step by 7- $\alpha$ -dehydroxylase (which converts unconjugated primary bile acids into secondary bile acids).<sup>18,19</sup> Recent studies demonstrated that the activity of bile-metabolizing enzymes (7- $\alpha$ -dehydroxylase in particular) is partly protective against CDI in rodents.<sup>20,21</sup> Therefore, one hypothesis has been as to whether patients with rCDI – with antibiotic-mediated destruction of their gut microbiota – are deficient in gut microbiota members which produce bile-metabolizing enzymes, with the consequent enrichment in TCA (promoting *C. difficile* germination) and loss of DCA (facilitating vegetative growth) perpetuating active disease. By extension, FMT may restore bacteria that produce these enzymes, and reverse the abnormal bile acid *milieu* of the distal gut.

Supporting this hypothesis, the stool bile acid *milieu* is enriched in TCA in human patients with rCDI, whilst secondary bile acids predominate in post-FMT stool.<sup>22–25</sup> Similarly, healthy donor stool contains little TCA, but relatively high levels of secondary bile acids.<sup>22–24</sup> Further recent work from our laboratories explored the dynamics of microbial bile acid-metabolizing enzymes in patients with rCDI, and the impact of FMT upon this. Predicted *bsh* gene abundance was significantly reduced in patients with rCDI, compared to patients with a primary episode of CDI, and/or

healthy controls.<sup>24</sup> Furthermore, the stool microbiota from patients pre-FMT had a greatly reduced relative abundance of a broad range of BSH-producing bacteria compared to the stool of patients treated successfully with FMT and/or healthy donors.<sup>23</sup> Successful FMT for rCDI rapidly and sustainably restored stool *bsh* gene copy number and BSH functional activity from the almost negligible levels found pre-FMT up to comparably high levels to that found in healthy donors.<sup>23</sup> Finally, stool *C. difficile* counts were ~70% reduced in an rCDI mouse model after administration of *E. coli* expressing highly-active BSH relative to mice administered BSH-negative *E. coli*.<sup>23</sup> Collectively, these data are strongly supportive that FMT-mediated restoration of gut microbial bile acid metabolism – and particularly BSH functionality – is a key mechanism underlying FMT's efficacy in rCDI. An additional recent relevant finding has been that gut bacteria expressing 7- $\alpha$ -dehydroxylase are also able to produce tryptophan-derived antibiotics, which themselves inhibit the cell division of *C. difficile*.<sup>26</sup>

In addition to the direct action of bile acids upon *C. difficile*, recent findings hint at complementary mechanisms by which gut microbiota-bile acid interactions contribute to protection from CDI. For example, successful FMT for rCDI was also found to be associated with increased circulating fibroblast growth factor (FGF)-19, consistent with the upregulation of the farnesoid X receptor (FXR)-FGF pathway.<sup>27</sup> In line with this, pre-treatment with the tertiary bile acid ursodeoxycholic acid (UDCA) in a CDI mouse model was associated with increased FXR-FGF signaling-related transcripts and attenuated inflammation.<sup>28</sup> Furthermore, upregulated FXR after FMT for rCDI may contribute to the resolution of the colitis induced by *C. difficile*, since FXR agonist administration in a rodent colitis model was associated with reduced colonic inflammation and a more intact intestinal barrier.<sup>29</sup> Ileal FXR activation also causes reduced hepatic bile acid synthesis by negative feedback; this may result in reduced TCA secretion into the gut, further reducing germination of *C. difficile*. Additionally, microbially-mediated production of certain secondary bile acids has been demonstrated to promote the generation of peripheral regulatory T cells,<sup>30</sup> linking these metabolites with colonic immunity.

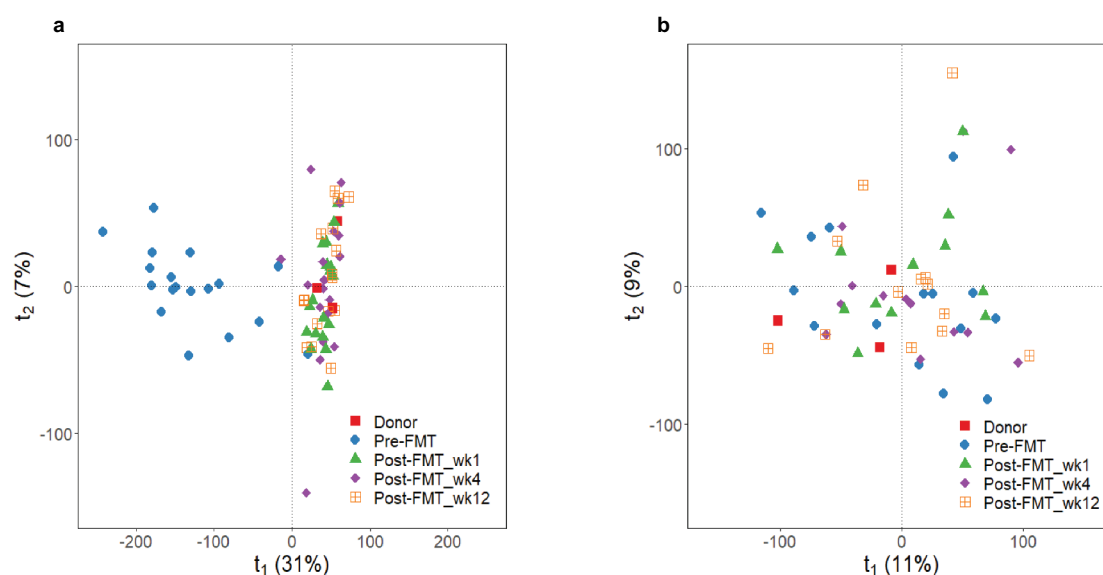
## 2.2. Short chain fatty acid metabolism

One further group of metabolites well-studied in this field are the short chain fatty acids (SCFAs). The major source of these is bacterial fermentation of partially and non-digestible carbohydrates (primarily dietary), although certain amino acids may also be a source.<sup>31</sup> In rodent studies, while antibiotics reduced SCFA levels in stool, higher SCFA levels were found to be associated with protection from *C. difficile* growth, suggesting an interplay between antibiotics, SCFAs, and rCDI risk.<sup>17</sup>

Human studies have demonstrated that levels of a range of SCFAs within stool (including acetate, propionate and butyrate) are very low in rCDI, but restored to levels comparable to healthy donors after successful FMT.<sup>25</sup> However, given the close link between antibiotic use, dietary intake and SCFA production, it is difficult to ascertain from human observational studies alone whether the post-FMT increase in stool SCFAs reflects changes in dietary intake and/or recovery after antibiotic discontinuation post-resolution of CDI, or whether this is driven by specific FMT-related gut microbiota changes.

As such, our laboratory investigated this using an artificial gut ("chemostat") model of CDI, whereby the confounding factor of variable dietary intake was removed.<sup>32</sup> In these experiments, recovery of the levels of certain SCFAs (including butyrate) was observed after cessation of antibiotics and even prior to FMT, consistent with spontaneous gut microbiota recovery. However, levels of valerate (the five carbon SCFA) only recovered to baseline levels after the administration of FMT, and not after antibiotic cessation alone. Valerate caused a dose-dependent inhibition of the vegetative growth of several *C. difficile* ribotypes, but no adverse growth effects upon several different common gut commensal bacteria. In a CDI mouse model, oral administration of valerate (in the form of glycerol trivalerate) resulted in a ~ 95% reduction in *C. difficile* stool counts compared to control-treated mice.<sup>32</sup> Additionally, successful FMT for rCDI in humans was associated with rapid, sustained restoration of stool valerate to similar levels as found in stool donors.<sup>32</sup>

Restoration of SCFAs by FMT may benefit patients recovering from CDI by mechanisms



**Figure 1.** Metabolic profile differences in donors and recipients before and after FMT. Principal component analysis (PCA) scores plots of  $^1\text{H}$ -NMR spectra from fecal water (a) and urine (b) samples from donors ( $n = 3$ ) and recipients collected at different timepoints (for stool recipient samples:  $n = 18$  for timepoints 0 (pre-FMT), 1, 4 and  $n = 16$  for timepoint 12; for urine:  $n = 15$  for timepoint 0,  $n = 12$  for timepoint 1,  $n = 13$  for timepoints 4 and 12).

beyond inhibiting the growth of *C. difficile*, including a possible role in the resolution of colitis and gut barrier function. For instance, in a mouse model of CDI, butyrate was demonstrated to improve intestinal barrier function and reduce intestinal inflammation via a mechanism involving activation of the transcription factor HIF-1.<sup>33</sup> Furthermore, SCFAs regulate the size and function of the colonic regulatory T cell population in mice, which has been directly demonstrated to be a protective mechanism against colitis.<sup>34</sup> SCFAs (including valerate) are also recognized to inhibit histone deacetylases (HDACs), with the resultant change in transcription of a range of genes having a net anti-inflammatory effect on the host immune phenotype.<sup>35,36</sup> Of particular relevance, exogenous valerate has already been shown to ameliorate chemically-induced colitis in a rodent model via HDAC inhibition.<sup>37</sup>

### 3. Impact of FMT for rCDI upon gut microbial metabolites: novel areas

To investigate further metabolic pathways that FMT for rCDI may impact upon, we performed proton nuclear magnetic resonance spectroscopy ( $^1\text{H}$ -NMR) on stool (fecal water) and urine from rCDI patients participating in a clinical trial comparing capsule to colonoscopic delivery of FMT, with

samples collected prior to FMT, and at weeks 1, 4, and 12 post-FMT.<sup>38</sup> Samples from 18 patients and 3 donors were analyzed; further details on methodology are provided in the **Supplementary Material**.  $^1\text{H}$ -NMR is an ‘information-rich’ analytical technique used in metabolomics and popular for its capability to simultaneously detect many biochemical entities (host and microbial) from complex biofluids in a nondestructive manner.<sup>39</sup>

Global metabolic profiling of fecal water changed notably after FMT, with pre-FMT samples clustering apart from post-FMT and donor samples across the first principal component in the principal components analysis (PCA) scores plot (Figure 1). This indicates that patients acquired a stool metabolic profile more similar to donors already in the first week post-FMT, and this was maintained for 12 weeks. We did not observe any strong effect of the FMT mode on the metabolic profile (Supplementary Figure 1a). To assess the proportion of features changing after FMT, we used mixed effects models<sup>40</sup> with time as categorical variable and using our first time point (pre-FMT) as reference category. Specifically, we tested the time effect as a likelihood ratio test among two models – with or without time as the predictor – fitted to each NMR feature (binned spectra intensities):  $\text{NMR feature} \sim \text{time} + \text{FMT mode} + (1|\text{Donor}) + (1|$



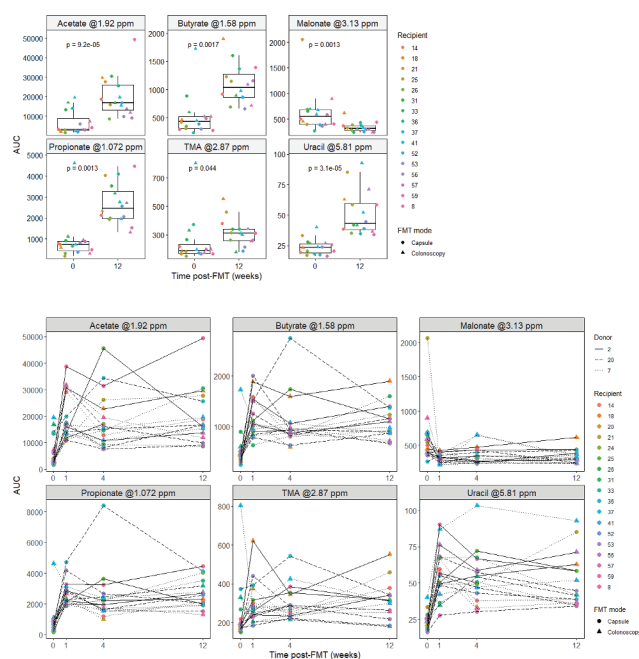
Recipient). A total of 15,162 out of 21,252 ( $\approx 71\%$ ) features from fecal water changed with time, which agrees with the clear clustering already observed in the PCA (Figure 1(a)). Using statistical total correlation spectroscopy (STOCSY),<sup>41</sup> 2D J-resolved (JRES)<sup>1</sup>H-NMR spectra and NMR peak databases, we annotated different metabolites and calculated the area under the curve (AUC) of a representative peak for each, using spectra normalized by probabilistic quotient normalization (PQN).<sup>42</sup>

In stool, the SCFAs acetate, butyrate and propionate increased 4.96, 2.46 and 3.46 median fold at 12 weeks post-FMT, respectively (Figure 2), consistent with previous findings, as described above. Further analysis and interpretation regarding the effect of FMT upon SCFAs (as well as upon bile acids) is provided within the **Supplementary Material**. Interestingly, we also detected a 1.7-fold increase in the microbial metabolite trimethylamine (TMA; produced by gut bacteria from dietary choline and L-carnitine), a 1.95-fold increase in the pyrimidine uracil, and a 0.55-fold decrease in the carboxylic acid malonate (Figure 2). To our knowledge, this is the first time an increase in TMA is

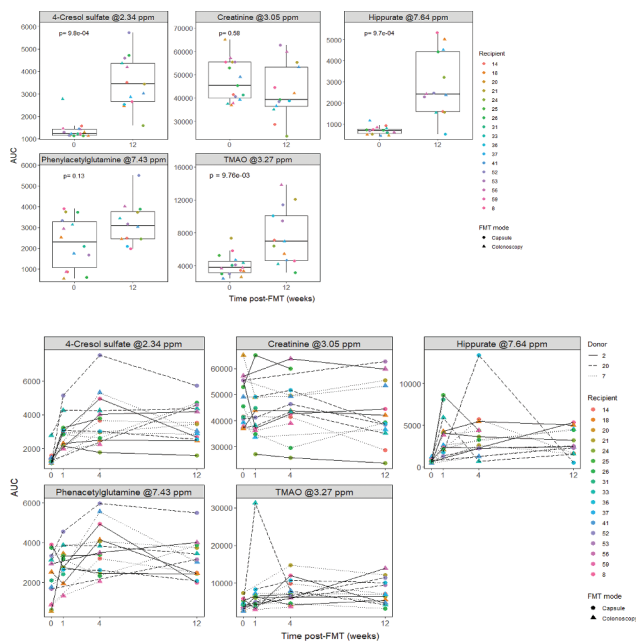
reported following FMT intervention in patients with rCDI. Similarly, an increase in uracil has not been reported previously, although a decrease in this metabolite has been observed in feces from mice treated with vancomycin;<sup>43</sup> as such, the observed uracil increase could be to at least partly a proxy of gut ecology recovery after cessation of vancomycin, rather than an effect of FMT *per se*. The decrease in malonate after FMT has also not been described before. The capacity to metabolize malonate by the gut microbiota and its impact on host metabolism has not previously been characterized in detail, but appears to be negatively correlated with acetate across different experimental landscapes,<sup>44,45</sup> and could be associated with an increase in gut bacteria using this metabolite as energy source.

In urine, PCA scores did not show clear clusters among different groups or FMT modes (Figure 1(b) and **Supplementary Figure 1b**), but 3,099 out of 27,271 features ( $\approx 11\%$ ) changed with time. Particularly, we found a 3.46-fold increase in the microbial-host co-metabolite hippurate (produced by conjugation of the microbial compound benzoate with glycine in the liver), a 1.85-fold increase in the co-metabolite trimethylamine-N-oxide (TMAO; resulting from TMA oxidation in the liver), and a 2.8-fold increase in 4-cresol sulfate (4-CS; the hepatic sulfonation of microbial 4-cresol). Finally, we also found a 1.34-fold increase in phenylacetylglutamine (PAG) (although this was not significant at the 5% significance level), while no differences were found in creatinine (Figure 3). As with fecal uracil, the observed increases in hippurate and PAG could reflect recovery of gut microbial ecology after vancomycin cessation, as those two metabolites were also reduced in urine from mice treated with vancomycin.<sup>43</sup>

The increase in urinary TMAO correlates with the observed increase in fecal TMA (Figure 2), suggesting a higher availability of TMA for oxidation in the liver. This could also be a consequence of microbial restoration after FMT, as the previous study shows an increase in urine TMAO in control mice as compared to those treated with antibiotic. TMA and TMAO synthesis is markedly altered after antibiotic treatment in humans, with gut microbial production of TMA from L-carnitine



**Figure 2.** Metabolite changes in fecal water. Area under curve (AUC) of metabolite representative peaks at 0 (pre-FMT) and 12 weeks post-FMT (top;  $n = 18$  for timepoint 0 and  $n = 16$  for timepoint 12), and across all measured time points (bottom;  $n = 18$  for timepoints 0, 1, 4 and  $n = 16$  for timepoint 12).  $P$ -values were calculated using paired Wilcoxon signed rank test.



**Figure 3.** Metabolite changes in urine. AUC of metabolite representative peaks at 0 (pre-FMT) and 12 weeks post-FMT (top;  $n = 15$  for timepoint 0 and  $n = 13$  for timepoint 12), and across all measured time points (bottom;  $n = 15$  for timepoint 0,  $n = 12$  for timepoint 1,  $n = 13$  for timepoints 4 and 12).  $P$ -values were calculated using paired Wilcoxon signed rank test.

appearing to involve multiple commensal bacteria (only partially identified) rather than a single community member.<sup>46</sup> However, given the association of TMAO with atherosclerosis,<sup>47</sup> long-term effects of FMT on TMA and TMAO levels in recipient patients deserves further investigation, as well as potentially the need to triage donors according to their TMA or TMAO levels; this is particularly of relevance given the interest of the potential role of FMT in treating obesity/metabolic syndrome (MetS). Given that a vegan diet is associated with reduced capacity to synthesize TMAO on the so-called ‘carnitine challenge’<sup>47</sup> Smits and colleagues performed a pilot study where 20 male MetS patients were randomized to either receive lean vegan donor FMT or autologous FMT.<sup>48</sup> Interestingly, while certain gut microbiota changes were seen in the vegan donor FMT recipients, there was neither an improvement of arterial wall inflammation, nor a change in TMA/TMAO metabolism, by two weeks post-FMT.<sup>48</sup> The value of vegan FMT donors for MetS treatment – together with patient diet and exercise patterns post-FMT – needs to be further evaluated in larger cohorts, and its impact on TMA/TMAO metabolism and associated gut

microbes further explored using ‘confounder-reduced’ batch cultures and/or mouse models. Additionally, a clinically-applicable means of assessing the ability of the gut microbiota to produce TMAO – through the oral carnitine challenge test<sup>49</sup> – may be a useful test for screening of donors in the future.

The increase in 4-CS post-FMT is somewhat less expected, given that 4-cresol is produced by *C. difficile*.<sup>50</sup> 4-CS has been associated with inflammation and a range of diseases, including kidney failure, autism, and colorectal cancer.<sup>51,52</sup> The lack of change in creatinine levels after FMT (Figure 3) indicates that kidney function does not appear to be affected by the intervention. As such, one possible explanation may be that FMT disrupts the life cycle of *C. difficile* without necessarily causing its clearance from the gut. Another explanation for the increase in urinary 4-CS is that it may reflect increased bacterial production of 4-cresol after FMT, restored by any of the number of non-*C. difficile* gut bacteria which also produce this metabolite,<sup>53</sup> with the possible concomitant shift toward increased consumption of proteins as the patient symptoms improve. This would provide the gut microbiota higher amounts of amino acid tyrosine, the 4-cresol substrate. However, detailed dietary data in our study was not available, which is a limitation of this study when interpreting changes in TMAO and 4-cresol. As with TMAO, the long-term impact of FMT on 4-CS levels, and possible causes for its increase other than rCDI – or whether it relates to a higher risk of recurrence – remains to be assessed.

#### 4. Conclusions

Our past work and the novel results presented in this *Addendum* suggest a strong impact of FMT on the metabolic profile of recipients soon after intervention. Mechanistic insights using mouse models, chemostats or human samples on the role of these metabolites during rCDI could give us further insight into the pathogenesis of rCDI, biomarkers of FMT outcomes, and – of greatest clinical relevance – the potential to replace FMT with a more refined microbial therapeutic, e.g. a ‘cocktail’ of purified BSH and glycerol trivalerate. Furthermore, consequences

of such metabolic shifts, especially at later follow-up time points and controlling for dietary factors, need further investigation, and may be of particular significance as FMT usage extends beyond CDI and into other diseases.

## Addendum to:

McDonald J.A.K., Mullish B.H., Pechlivanis A., Liu Z., Brignardello J., Kao D., Holmes E., Li J.V., Clarke T.B., Thursz M.R., Marchesi J.R. Inhibiting growth of *Clostridioides difficile* by restoring valerate, produced by the intestinal microbiota. *Gastroenterology* (2018); 155(5):1495-1507.e15

Monaghan T., Mullish B.H., Patterson J., Wong G.K., Marchesi J.R., Xu H., Jilani T., Kao D. Effective fecal microbiota transplantation for recurrent *Clostridioides difficile* infection in humans is associated with increased signalling in the bile acid-farnesoid X receptor-fibroblast growth factor pathway. *Gut Microbes* (2019);10(2):142-148. doi: 10.1080/19490976.2018.1506667.

Mullish B.H., McDonald J.A.K., Pechlivanis A., Allegretti J. R., Kao D., Barker G.B., Kapila D., Petrof D., Joyce S.A., Gahan G.M., Glegola-Madesjska I., Williams H.R.T., Holmes E., Clarke T.B., Thursz M.R., Marchesi J.R. Microbial bile salt hydrolases mediate the efficacy of faecal microbiota transplant in the treatment of recurrent *Clostridioides difficile* infection. *Gut* (2019); 68(10):1791-1800. doi: 10.1136/gutjnl-2018-317842

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## Disclosures statement













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## References

1. McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, Dubberke ER, Garey KW, C V G, Kelly C, et al. Clinical practice guidelines for clostridium difficile infection in adults and children: 2017 update by the infectious diseases society of America (IDSA) and society for healthcare epidemiology of America (SHEA). *Clin Infect Dis*. [Internet] 2018 [cited 2018 Feb 22]; 31:431–455. Available from: <https://academic.oup.com/cid/advance-article/doi/10.1093/cid/cix1085/4855916>.
2. Mullish BH, Quraishi MN, Segal JP, McCune VL, Baxter M, Marsden GL, Moore DJ, Colville A, Bhala N, Iqbal TH, et al. The use of faecal microbiota transplant as treatment for recurrent or refractory *Clostridium difficile* infection and other potential indications: joint British society of gastroenterology (BSG) and Healthcare Infection Society (HIS) guidelines. *Gut*. [Internet] 2018 [cited 2018 Sep 5]; 67:1920–1941. Available from: <http://gut.bmj.com/lookup/doi/10.1136/gutjnl-2018-316818>.



3. Allegretti JR, Mullish BH, Kelly C, Fischer M. The evolution of the use of faecal microbiota transplantation and emerging therapeutic indications. *Lancet*. [Internet] 2019 [cited 2019 Aug 5]; 394:420–431. Available from: [https://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(19\)31266-8/fulltext#](https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(19)31266-8/fulltext#).
4. DeFilipp Z, Bloom PP, Soto MT, Mansour MK, Sater MRA, Huntley MH, Turbett S, Chung RT, Chen Bin Y, Hohmann EL. Drug-resistant *e. coli* bacteremia transmitted by fecal microbiota transplant. *N Engl J Med*. [Internet] 2019 [cited 2019 Nov 16]; 381:2043–2050. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/31665575>.
5. Duvallet C, Zellmer C, Panchal P, Budree S, Osman M, Alm EJ. Framework for rational donor selection in fecal microbiota transplant clinical trials. *PLoS One*. [Internet] 2019 [cited 2020 Apr 29]; 14:e0222881. Available from: <http://dx.plos.org/10.1371/journal.pone.0222881>.
6. Smillie CS, Sauk J, Gevers D, Friedman J, Sung J, Youngster I, Hohmann EL, Staley C, Khoruts A, Sadowsky MJ, et al. Strain tracking reveals the determinants of bacterial engraftment in the human gut following fecal microbiota transplantation. *Cell Host Microbe*. [Internet] 2018 [cited 2019 Feb 27]; 23:229–240.e5. Available from: <https://www.sciencedirect.com/science/article/pii/S1931312818300386?via%3Dihub>.
7. Staley C, Kaiser T, Vaughn BP, Graiziger CT, Hamilton MJ, Khoruts A, Sadowsky MJ, Vaughn BP, Graiziger CT, Hamilton MJ, et al. Predicting recurrence of *Clostridium difficile* infection following encapsulated fecal microbiota transplantation. *Microbiome*. [Internet] 2018 [cited 2018 Oct 6]; submitted:166. Available from: <https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-018-0549-6>.
8. Petrof EO, Gloor GB, Vanner SJ, Weese SJ, Carter D, Daigneault MC, Brown EM, Schroeter K, Allen-Vercoe E. Stool substitute transplant therapy for the eradication of *Clostridium difficile* infection: “RePOOPulating” the gut. *Microbiome*. [Internet] 2013 [cited 2017 Oct 3]; 1:3. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24467987>.
9. Khanna S, Pardi DS, Kelly CR, Kraft CS, Dhore T, Henn MR, Lombardo M-J, Vulic M, Ohsumi T, Winkler J, et al. A novel microbiome therapeutic increases gut microbial diversity and prevents recurrent *Clostridium difficile* infection. *J Infect Dis*. [Internet] 2016 [cited 2017 Oct 3]; 214:173–181. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26908752>.
10. Ott SJ, Waetzig GH, Rehman A, Moltzau-Anderson J, Bharti R, Grasis JA, Cassidy L, Tholey A, Fickenscher H, Seegert D, et al. Efficacy of sterile fecal filtrate transfer for treating patients with *clostridium difficile* infection. *Gastroenterology*. [Internet] 2017 [cited 2017 Jun 9]; 152:799–811.e7. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0016508516353549>.
11. Zuo T, Wong SH, Cheung CP, Lam K, Lui R, Cheung K, Zhang F, Tang W, Ching JYL, Wu JCY, et al. Gut fungal dysbiosis correlates with reduced efficacy of fecal microbiota transplantation in *Clostridium difficile* infection. *Nat Commun*. [Internet] 2018 [cited 2019 Feb 22]; 9:3663. Available from: <http://www.nature.com/articles/s41467-018-06103-6>.
12. Draper LA, Ryan FJ, Smith MK, Jalanka J, Mattila E, Arkkila PA, Ross RP, Satokari R, Hill C. Long-term colonisation with donor bacteriophages following successful faecal microbial transplantation. *Microbiome*. [Internet] 2018 [cited 2018 Dec 21]; 6:220. Available from: <https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-018-0598-x>.
13. Zuo T, Wong SH, Lam K, Lui R, Cheung K, Tang W, Ching JYL, Chan PKS, Chan MCW, Wu JCY, et al. Bacteriophage transfer during faecal microbiota transplantation in *Clostridium difficile* infection is associated with treatment outcome. *Gut*. [Internet] 2017 [cited 2017 Oct 7]; gutjnl-2017-313952. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28539351>.
14. Park H, Laffin MR, Jovel J, Millan B, Hyun JE, Hotte N, Kao D, Madsen KL. The success of fecal microbial transplantation in *Clostridium difficile* infection correlates with bacteriophage relative abundance in the donor: a retrospective cohort study. *Gut Microbes*. [Internet] 2019 [cited 2020 May 12]; 10:676–687. Available from: <https://www.tandfonline.com/doi/full/10.1080/19490976.2019.1586037>.
15. Sorg JA, Sonenshein AL. Bile salts and glycine as cogerminants for *clostridium difficile* spores. *J Bacteriol*. [Internet] 2008 [cited 2017 Oct 7]; 190:2505–2512. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18245298>.
16. Sorg JA, Sonenshein AL. Inhibiting the initiation of *clostridium difficile* spore germination using analogs of chenodeoxycholic acid, a bile acid. *J Bacteriol*. [Internet] 2010 [cited 2018 Jan 16]; 192:4983–4990. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20675492>.
17. Theriot CM, Koenigsnecht MJ, Carlson PE, Hatton GE, Nelson AM, Li B, Huffnagle GB, Li ZJ, Young VB, Bowman AA, et al. Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to *Clostridium difficile* infection. *Nat Commun*. [Internet] 2014 [cited 2017 Oct 7]; 5:3114. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4863611/pdf/sph0046.pdf>.
18. Jones BV, Begley M, Hill C, Gahan CGM, Marchesi JR. Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. *Proc Natl Acad Sci U S A*. [Internet] 2008 [cited 2017 Oct 7]; 105:13580–13585. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18757757>.
19. Ridlon JM, Kang D-J, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. *J Lipid Res*. [Internet] 2006 [cited 2017 Oct 7]; 47:241–259. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16299351>.

20. Buffie CG, Bucci V, Stein RR, McKenney PT, Ling L, Goble A, No D, Liu H, Kinnebrew M, Viale A, et al. Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile*. *Nature*. [Internet] 2014 [cited 2017 Oct 7]; 517:205–208. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/25337874>.
21. Studer N, Desharnais L, Beutler M, Brugiroux S, Terrazos MA, Menin L, Schürch CM, McCoy KD, Kuehne SA, Minton NP, et al. Functional intestinal bile acid 7 $\alpha$ -dehydroxylation by *Clostridium scindens* associated with protection from *Clostridium difficile* infection in a gnotobiotic mouse model. *Front Cell Infect Microbiol*. [Internet] 2016 [cited 2018 May 2]; 6:191. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/28066726>.
22. Weingarden AR, Chen C, Bobr A, Yao D, Lu Y, Nelson VM, Sadowsky MJ, Khoruts A. Microbiota transplantation restores normal fecal bile acid composition in recurrent *Clostridium difficile* infection. *Am J Physiol Liver Physiol*. 2014;306:G310–9.
23. Mullish BH, McDonald JAKK, Pechlivanis A, Allegretti JR, Kao D, Barker GF, Kapila D, Petrof EO, Joyce SA, Gahan CGMM, et al. Microbial bile salt hydrolases mediate the efficacy of faecal microbiota transplant in the treatment of recurrent *Clostridioides difficile* infection. *Gut*. [Internet] 2019 [cited 2019 Feb 11]; 68:1791–1800. Available from: <https://gut.bmj.com/content/early/2019/02/11/gutjnl-2018-317842>.
24. Allegretti JR, Kearney S, Li N, Bogart E, Bullock K, Gerber GK, Bry L, Clish CB, Alm E, Korzenik JR. Recurrent *Clostridium difficile* infection associates with distinct bile acid and microbiome profiles. *Aliment Pharmacol Ther*. [Internet] 2016 [cited 2017 Oct 7]; 43:1142–1153. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27086647>.
25. Seekatz AM, Theriot CM, Rao K, Chang Y-M, Freeman AE, Kao JY, Young VB. Restoration of short chain fatty acid and bile acid metabolism following fecal microbiota transplantation in patients with recurrent *Clostridium difficile* infection. *Anaerobe*. [Internet] 2018 [cited 2019 Mar 4]; 53:64–73. Available from: <https://www.sciencedirect.com/science/article/pii/S1075996418300581#bib52>.
26. Kang JD, Myers CJ, Harris SC, Kakiyama G, Lee I-K, Yun B-S, Matsuzaki K, Furukawa M, Min H-K, Bajaj JS, et al. Bile acid 7 $\alpha$ -dehydroxylating gut bacteria secrete antibiotics that inhibit *Clostridium difficile*: role of secondary bile acids. *Cell Chem Biol*. [Internet] 2019 [cited 2019 Mar 9]; 26:27–34.e4. Available from: <https://www.sciencedirect.com/science/article/pii/S2451945618303350>.
27. Monaghan T, Mullish BH, Patterson J, Wong GKSK, Marchesi JR, Xu H, Jilani T, Kao D. Effective fecal microbiota transplantation for recurrent *Clostridioides difficile* infection in humans is associated with increased signalling in the bile acid-farnesoid X receptor-fibroblast growth factor pathway. *Gut Microbes*. [Internet] 2019 [cited 2018 Sep 9]; 10:1–7. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/30183484>.
28. Winston JA, Rivera AJ, Cai J, Thanissery R, Montgomery SA, Patterson AD, Theriot CM. Ursodeoxycholic acid (udca) mitigates the host inflammatory response during *Clostridioides difficile* infection by altering gut bile acids. *Infect Immun*. [Internet] 2020 [cited 2020 Jul 8]; 88. Available from: <https://pubmed.ncbi.nlm.nih.gov/32205405/>.
29. Gadaleta RM, van Erpecum KJ, Oldenburg B, Willemsen ECL, Renooij W, Murzilli S, Klomp LWJ, Siersema PD, Schipper MEI, Danese S, et al. Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. *Gut*. [Internet] 2011 [cited 2019 Mar 7]; 60:463–472. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21242261>.
30. Campbell C, McKenney PT, Konstantinovskiy D, Isaeva OI, Schizas M, Verter J, Mai C, Jin W-B, Guo C-J, Violante S, et al. Bacterial metabolism of bile acids promotes generation of peripheral regulatory T cells. *Nat*. 2020;2020:1–5.
31. Cook S. Review article: short chain fatty acids in health and disease. *Aliment Pharmacol Ther*. [Internet] 1998 [cited 2019 Feb 24]; 12:499–507. doi:10.1046/j.1365-2036.1998.00337.x.
32. McDonald JAK, Mullish BH, Pechlivanis A, Liu Z, Brignardello J, Kao D, Holmes E, Li JV, Clarke TB, Thursz MR, et al. Inhibiting growth of *Clostridioides difficile* by restoring valerate, produced by the intestinal microbiota. *Gastroenterology*. [Internet] 2018; 155:1495–1507.e15. doi:10.1053/j.gastro.2018.07.014.
33. Fachi JL, De Felipe JS, Pral LP, Da Silva BK, Corrêa RO, De Andrade MCP, Da Fonseca DM, Basso PJ, Câmara NOS, De Sales E Souza ÉL, et al. Butyrate protects mice from *Clostridium difficile*-induced colitis through an HIF-1-dependent mechanism. *Cell Rep*. [Internet] 2019 [cited 2020 Jul 8]; 27:750–761.e7. Available from: <https://pubmed.ncbi.nlm.nih.gov/30995474/>.
34. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M, Glickman JN, Garrett WS. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Sci* (80-). [Internet] 2013 [cited 2019 Mar 6]; 341:569–573. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23828891>.
35. Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. The role of short-chain fatty acids in health and disease. *Adv Immunol*. [Internet] 2014 [cited 2019 Mar 7]; 121:91–119. Available from: <https://www.sciencedirect.com/science/article/pii/B9780128001004000039?via%3Dihub>.
36. Yuille S, Reichardt N, Panda S, Dunbar H, Mulder IE. Human gut bacteria as potent class I histone deacetylase inhibitors in vitro through production of butyric acid and valeric acid. *PLoS One*. [Internet] 2018 [cited 2019 Mar 7]; 13:e0201073. Available from: <https://dx.plos.org/10.1371/journal.pone.0201073>.

37. Glauben R, Batra A, Fedke I, Zeitz M, Lehr HA, Leoni F, Mascagni P, Fantuzzi G, Dinarello CA, Siegmund B. Histone hyperacetylation is associated with amelioration of experimental colitis in mice. *J Immunol*. [Internet] 2006 [cited 2019 Mar 14]; 176:5015–5022. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16585598>.
38. Kao D, Roach B, Silva M, Beck P, Rioux K, Kaplan GG, Chang HJ, Coward S, Goodman KJ, Xu H, et al. Effect of oral capsule- vs colonoscopy-delivered fecal microbiota transplantation on recurrent *Clostridium difficile* infection: A randomized clinical trial. *JAMA - J Am Med Assoc*. [Internet] 2017 [cited 2017 Dec 12]; 318:1985–1993. doi:10.1001/jama.2017.17077.
39. Nicholson JK, Connelly J, Lindon JC, Holmes E. Metabonomics: A platform for studying drug toxicity and gene function. *Nat Rev Drug Discov*. 2002;1:153–161. doi:10.1038/nrd728.
40. Bates D, Mächler M, Bolker BM, Walker SC. Fitting linear mixed-effects models using lme4. *J Stat Softw*. 2015;67:1–48. doi:10.18637/jss.v067.i01.
41. Cloarec O, Dumas ME, Craig A, Barton RH, Trygg J, Hudson J, Blancher C, Gauguier D, Lindon JC, Holmes E, et al. Statistical total correlation spectroscopy: an exploratory approach for latent biomarker identification from metabolic 1H NMR data sets. *Anal Chem*. 2005;77:1282–1289. doi:10.1021/ac048630x.
42. Dieterle F, Ross A, Schlotterbeck G, Senn H. Probabilistic quotient normalization as robust method to account for dilution of complex biological mixtures. Application in 1H NMR metabonomics. *Anal Chem*. 2006;78:4281–4290. doi:10.1021/ac051632c.
43. Yap IKS, Li JV, Saric J, Martin FP, Davies H, Wang Y, Wilson ID, Nicholson JK, Utzinger J, Marchesi JR, et al. Metabonomic and microbiological analysis of the dynamic effect of vancomycin-induced gut microbiota modification in the mouse. *J Proteome Res*. 2008;7:3718–3728. doi:10.1021/pr700864x.
44. Zheng H, Yde CC, Clausen MR, Kristensen M, Lorenzen J, Astrup A, Bertram HC. Metabolomics investigation to shed light on cheese as a possible piece in the French paradox puzzle. *J Agric Food Chem*. 2015;63:2830–2839. doi:10.1021/jf505878a.
45. Luo L, Hu M, Li Y, Chen Y, Zhang S, Chen J, Wang Y, Lu B, Xie Z, Liao Q. Association between metabolic profile and microbiomic changes in rats with functional dyspepsia. *RSC Adv*. 2018;8:20166–20181. doi:10.1039/C8RA01432A.
46. Koeth RA, Lam-Galvez BR, Kirsop J, Wang Z, Levison BS, Gu X, Copeland MF, Bartlett D, Cody DB, Dai HJ, et al. L-Carnitine in omnivorous diets induces an atherogenic gut microbial pathway in humans. *J Clin Invest*. 2019;129(1):373–387.
47. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, Britt EB, Fu X, Wu Y, Li L, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med*. 2013;19:576–585. doi:10.1038/nm.3145.
48. Smits LP, Kootte RS, Levin E, Prodan A, Fuentes S, Zoetendal EG, Wang Z, Levison BS, Cleophas MCP, Kemper EM, et al. Effect of vegan fecal microbiota transplantation on carnitine- and choline-derived trimethylamine-N-oxide production and vascular inflammation in patients with metabolic syndrome. *J Am Heart Assoc*. [Internet] 2018 [cited 2020 May 7]; 7. Available from: <https://www.ahajournals.org/doi/10.1161/JAHA.117.008342>.
49. Wu WK, Chen CC, Liu PY, Panyod S, Liao BY, Chen PC, Kao HL, Kuo HC, Kuo CH, Chiu THT, et al. Identification of TMAO-producer phenotype and host-diet-gut dysbiosis by carnitine challenge test in human and germ-free mice. *Gut*. 2019;68:1439–1449. doi:10.1136/gutjnl-2018-317155.
50. Passmore IJ, Leterre MPM, Preston MD, Bianconi I, Harrison MA, Nasher F, Kaur H, Hong HA, Baines SD, Cutting SM, et al. Para-cresol production by *Clostridium difficile* affects microbial diversity and membrane integrity of Gram-negative bacteria. *PLoS Pathog*. 2018;14(9): e1007191.
51. Diether N, Willing B. Microbial fermentation of dietary protein: an important factor in diet-microbe-host interaction. *Microorganisms*. [Internet] 2019 [cited 2020 May 4]; 7:19. Available from: <http://www.mdpi.com/2076-2607/7/1/19>.
52. Persico AM, Napolioni V. Urinary p-cresol in autism spectrum disorder. *Neurotoxicol Teratol*. 2013;36:82–90. doi:10.1016/j.ntt.2012.09.002.
53. Saito Y, Sato T, Nomoto K, Tsuji H. Identification of phenol- and p-cresol-producing intestinal bacteria by using media supplemented with tyrosine and its metabolites. *FEMS Microbiol Ecol*. [Internet] 2018 [cited 2020 Jul 8]; 94. Available from: <https://pubmed.ncbi.nlm.nih.gov/29982420/>.